

DISPERSAL OF SPICEBUSH (*CALYCANTHUS OCCIDENTALIS*, CALYCANTHACEAE)
BY YELLOW JACKETS (GENUS *VESPULA*; HYMENOPTERA: VESPIDAE)

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ABSTRACT

Carnivorous wasps of the family Vespidae are known to seek out and disperse the diaspores of at least two North American and two Asian plant species. Attraction of the wasps to the diaspores is likely due to the release of volatile compounds that signal availability of an elaiosome rich in protein and fat, which the wasps remove before releasing the diaspore. It is thought that this interaction between carnivorous wasps and plants is rare, occurring in just a few plant species. Here, we present our findings on dispersal of spicebush (*Calycanthus occidentalis* Hook. & Arn.) achenes by carnivorous wasps of the genus *Vespula*. Observations and experiments were performed with the goals of discovering: how geographically widespread this interaction is; what the reward system is, if any; and, how wasps detect the achenes. Eight populations of *C. occidentalis* in northern California were used to observe wasps and plants, and to perform experiments on wasp attraction to the achenes. In all examined populations, workers of western yellowjacket (*Vespula pensylvanica* [de Saussure, 1857]) were observed entering mature *Calycanthus* receptacles, removing achenes, taking flight with them, and successfully transporting achenes through the air. Receptacles were found to open upward at an average angle of 45° (SD = 29°), preventing the achenes from falling to the ground when mature. No animals other than wasps were observed visiting the receptacles during the observations. Experiments suggest that wasps are attracted to an elaiosome-like organ of the achene. Nutritional analysis shows that this organ is high in fat and protein. Further experiments using solvent extracts of the achenes suggest that the attraction is likely mediated by volatile compounds.

Key Words: achene, California, fat, protein, reward, vespichochory, volatile.

The vast majority of insect-mediated plant diaspore dispersal is accomplished by ants (Handel and Beattie 1990; Hölldobler and Wilson 1990; Gómez and Espader 1998; Rico-Gray and Oliveira 2007; Lengyel et al. 2009) and is based on well-studied reward systems that usually involve a nutrient-rich accessory organ (elaiosome; Rico-Gray and Oliveira 2007). A less common and under-studied form of plant diaspore dispersal by insects is carried out by flying wasps and bees (Pellmyr 1985; Wallace and Trueman 1995; Jules 1996; Wallace et al. 2009; Zettler et al. 2001; Manohara 2013; Chen et al. 2018). Wasps, particularly those of the genera *Vespa* and *Vespula* (Vespidae) have been found to disperse the diaspores of four plant genera, *Aquilaria* Lam. (Thymelaeaceae; Manohara 2013), *Stemona* Lour. (Stemonaceae; Chen et al. 2018), *Trillium* L. (Melanthiaceae; Jules 1996; Zettler et al. 2001), and *Vancouveria* C.Morren & Decne. (Berberidaceae; Pellmyr 1985). Though the interaction is infrequently observed, its existence in four plant groups from divergent lineages on two continents suggests that it might occur in more groups of plants, and has escaped attention due to the past focus on ants as the major insect dispersers of diaspores.

Here, we report an investigation into wasp-based diaspore dispersal in spicebush, *Calycanthus occidentalis* Hook. & Arn. (Calycanthaceae). Spicebush is a California-endemic shrub and the only member of the Calycanthaceae found in western North America; the other members of the genus are in the southeastern U.S. and China, remnants of a disjunction that dates to the Miocene (Zhou et al. 2006). *Calycanthus occidentalis* is a shrub or small tree, most often found in riparian or other perennially moist microhabitats in the foothills (<1500 m) of the Sierra Nevada, Cascade Range, North Coast Range, and Klamath Mountains.

In the late spring to early summer, spicebush produces large (5–8 cm diameter) flowers, consisting of dark red tepals that open to reveal flattened stamens surrounding the opening of an invaginated, capsule-like receptacle. Individual ovaries are on the walls of this receptacle, and are probably pollinated by small beetles, which access the ovary chamber prior to full opening of the flower by crawling down a shaft produced by the inwardly-bent tips of the tepals (Grant 1950). The receptacle matures into a dry, capsule-like organ containing large (~1 cm long), closely packed achenes, each with a friable, reddish,

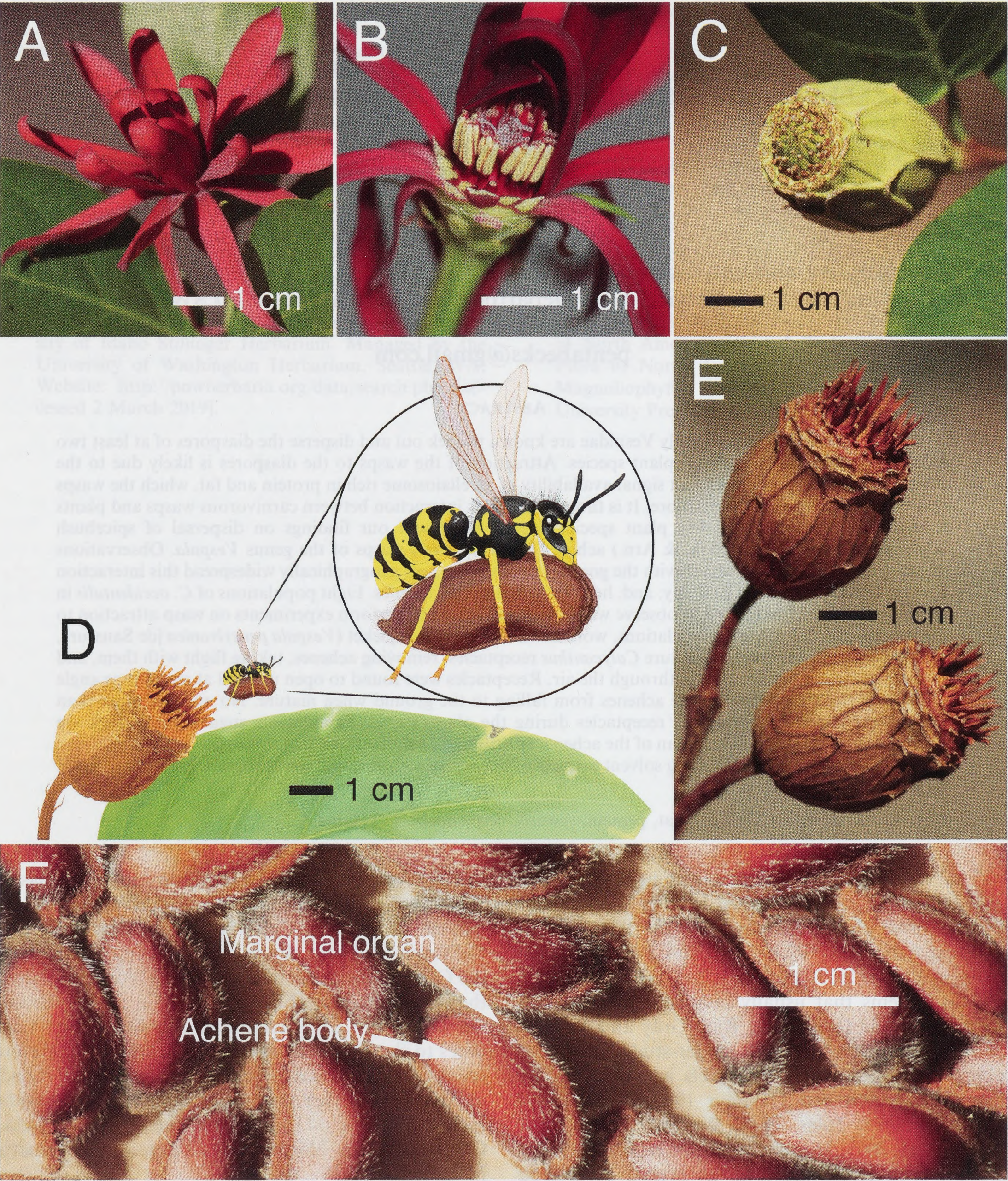


FIG. 1. Focal organisms of the study. (A) flower of spicebush, (B) developing receptacle of spicebush, (C) mature receptacle just prior to opening, (D) drawing of *Vespula pensylvanica* in flight with achene of spicebush (E) dry, open receptacle of spicebush, and (F) detail of achenes, showing the achene body and marginal organ.

annular organ (Fig. 1). Mature receptacles open from the tip, allowing access to the achenes (Fig. 1). Before the present work, nothing was known about dispersal of achenes in *C. occidentalis*.

In August 2016, initial observations were made of wasps (*Vespula pensylvanica* de Saussure, 1857)

visiting achenes placed in a bowl close to an open window at the residence of the first author's mother in the Butte Creek watershed, outside Chico, CA (Vanessa Church, personal communication). Examination of spicebush plants in this area revealed wasps visiting receptacles and removing achenes. As

this interaction between wasps and *C. occidentalis* was not previously reported, an investigation was launched to learn more about the system. Populations of *C. occidentalis* were visited to observe wasp activity, to collect data on receptacle morphology, and to obtain achenes for experiments and analysis of nutritional contents. Experiments were carried out at a subset of the eight focal populations, as well as at sites outside the geographic range of spicebush. The overall aims of this work were to determine: 1) how geographically widespread the interaction may be, 2) what the reward system is, if any, and 3) how wasps detect the achenes.

MATERIALS AND METHODS

In the summer of 2016, eight populations of *C. occidentalis* (Table 1; Fig. 2; Appendix I) were visited by the first author. These populations were selected to represent major population centers of the species (Consortium of California Herbaria 2018). Populations were accessed via vehicle and on foot. At each site, *Vespula* workers and their behavior in and around spicebush receptacles were observed. Observations were carried out for at least 3 hr at each site, and at least ten individual shrubs were examined. The goal of these observations was to assess whether *Vespula* are attracted to *Calycanthus* receptacles in the wild. If a single *Vespula* wasp was observed entering a *Calycanthus* receptacle, this was counted as a successful visitation event; other than confirming that the visitation occurred, no other data was collected; the number of visits was not quantified. At a subset of sites, measurements were taken of the orientation of the mature receptacles, to determine the likelihood that achenes would naturally fall from open receptacles to the ground. Measurements of the receptacle angles were taken using a home-made

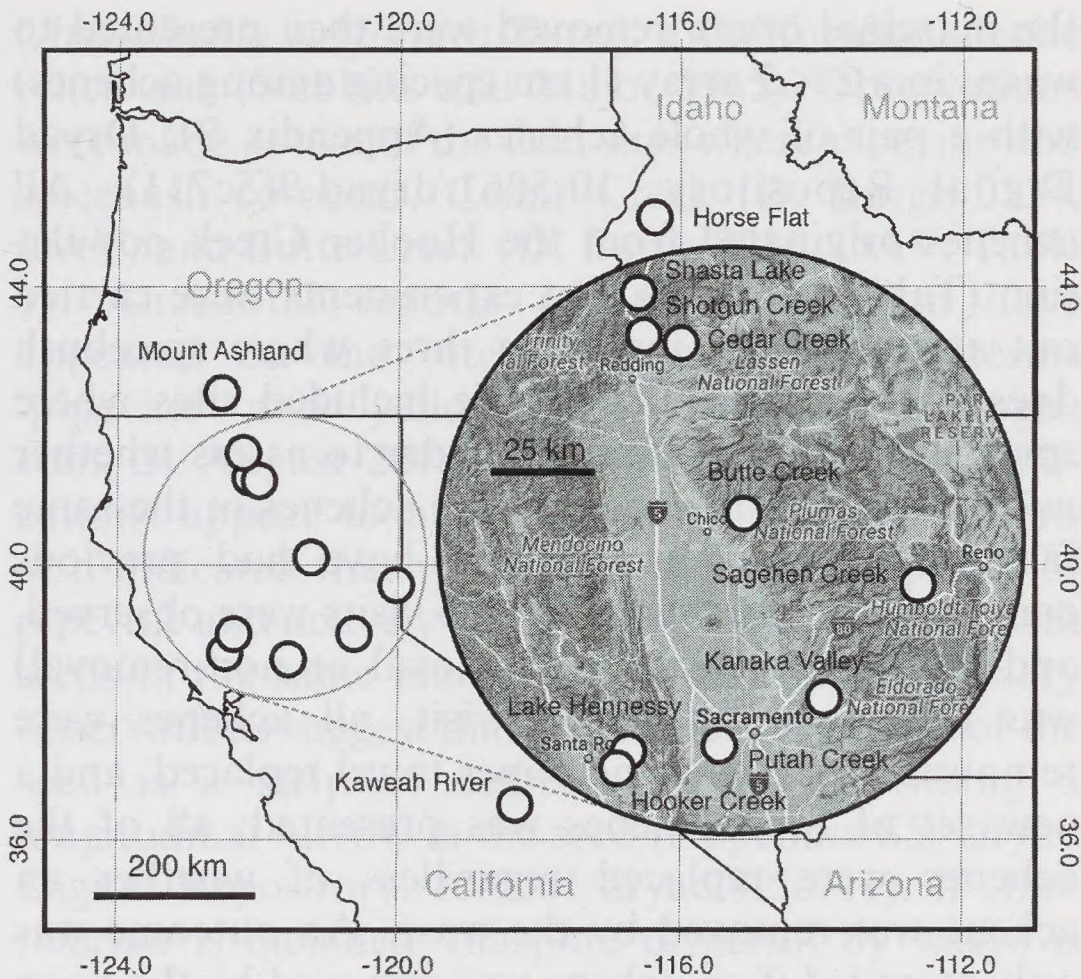


FIG. 2. Map of sites visited for this research.

inclinometer consisting of a protractor with a level and a plumb-bob attached. The angle was measured along the longitudinal axis of the receptacle, and recorded as the difference from horizontal (0°), with a maximum of 90° in either direction (i.e., a of range from −90° to 90°). At a subset of the sites, achenes were collected from open receptacles and allowed to dry in open paper bags for 12 hr before they were stored for later use in the same paper bags.

A series of experiments were carried out to determine: 1) whether wasps are attracted to the marginal organ or to the remaining part of the achene; 2) whether achenes are returned to the nest after collection by wasps; and 3) whether attraction to achenes is visual or olfactory. In the first experiment, the marginal organ was removed from a set of achenes using a razor. A pair of achenes with

TABLE 1. SITES VISITED FOR ACHENE COLLECTING, MEASUREMENTS, AND EXPERIMENTAL WORK. Plant voucher = collection code for spicebush voucher, and reference code for site; all voucher specimens were deposited at DAV (Appendix I). Wasp voucher = study-specific collection code for wasp voucher specimens; all specimens deposited at the U. S. Davis Bohart Museum of Entomology (Appendix II). Angle = sites at which the angles of the receptacle openings were measured. Mass = sites where achene mass was measured. Under Experiments, Choice indicates sites where achene choice experiments were carried out; Nest indicates sites where achenes were presented to nests and nests then excavated to determine if achenes were returned to the nest; Extract indicates source populations for achene extracts used in trapping experiments.

Site name	Plant voucher	Wasp voucher	Angle	Mass	Experiments		
					Choice	Nest	Extract
Butte Creek	D. Burge 2122	1000	X	X	X		X
Hooker Creek	D. Burge 2123	1005, 1006	X	X	X		
Sagehen Creek		1007, 1008			X	X	
Shasta Lake	D. Burge 2125	1010	X	X	X		
Shotgun Creek	D. Burge 2127	1011	X	X	X		
Mount Ashland		1012, 1013			X		
Cedar Creek	D. Burge 2132	1017	X		X		
Horse Flat		1019, 1020			X		
Putah Creek		1022				X	
Kanaka Valley		1023				X	
Kaweah River	D. Burge 2201						X
Lake Hennessy	D. Burge 2284						X

the marginal organ removed were then presented to wasps in a 2×2 array (1 cm spacing among achenes) with a pair of whole achenes (Appendix S1; Dryad Digital Repository: 10.5061/dryad.9f5c711). All achenes originated from the Hooker Creek population (Table 1; Fig. 2), but experiments were carried out at eight sites, including three where spicebush does not occur (Table 1). We included sites where spicebush does not occur in order to assess whether naïve wasps are attracted to the achenes in the same fashion as wasps that might have had previous contact with *Calycanthus*. Wasp visits were observed, and the outcome (achene removal or non-removal) was recorded. After each visit, all achenes were removed, the underlying paper towel replaced, and a new set of four achenes was presented; all of the achenes were replaced regardless of whether an achene was removed by the wasp. An outcome was only recorded if an achene was removed by the wasp from the experimental arena. Experiments were carried out for 2 hr at each site, between 1500–1800 hr (Table 1). To statistically test achene preference, we conducted a two-sided binomial test in R (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria). The binomial test explicitly treated each of the wasp visits as having only two possible outcomes: 1) achene removed or 2) achene not removed. The third possible outcome of a wasp visit, ‘no choice,’ was therefore treated as a nuisance parameter and not modeled. Finally, voucher specimens for each wasp species visiting the achenes were obtained at each site and deposited at the Bohart Museum of Entomology (Table 1; Appendix II).

In the second experiment, three sites were selected (Table 1). At each site, 100 intact achenes were presented in an open dish within 10 m of a single *V. pensylvanica* nest. After all the achenes were taken by wasps, the nest was subdued using carbon dioxide gas, and excavated. The number of achenes found in the nest, where they were located, and their condition, was recorded. Voucher wasp specimens were obtained from each nest and deposited at the Bohart Museum of Entomology (Table 1; Appendix II).

In the third experiment, an organic solvent was used to extract whole achenes, and the resulting extract was tested for its ability to attract wasps. We tested achenes from three sites (Table 1). For each of the three sites, 100 intact achenes were soaked in 100 mL analytical grade pentane for 12 hr. The extract was evaporated on a Whatman 1001-125 Qualitative Filter Paper Circle, and the filter paper placed in a RESCUE! Non-Toxic Yellowjacket Trap (Sterling International, Inc., Spokane, WA). A negative control, a filter paper circle from which 100 mL of pure pentane was evaporated, was used for comparison. The four traps (one for each site extract, plus control) were suspended 1 m apart on a rope tied between two trees. The traps were left for a 5 hr period in the afternoon (1300–1800 hr), then the

number of trapped wasps was counted. The experiment was repeated three times on three different days (21–23 August 2017), using a new overnight extraction on each occasion. All experiments were performed at the Butte Creek site (Table 1).

Because preliminary evidence suggested that the marginal organ of the achene was the part most attractive to wasps, nutritional analysis of this organ was carried out at the Michigan State University Diagnostic Center for Population and Animal Health. Variables measured included proportion of crude protein, crude fat, water-soluble carbohydrates, simple sugars, and starch (all on a dry mass basis). A total of 2 g of the achene marginal organ (Fig. 1) from the Hooker Creek population (Table 1; Fig. 2) was sent for analysis.

RESULTS

At all but two of the sites visited for observations (Table 1), workers of *V. pensylvanica* were seen hovering near the openings of mature receptacles, entering the receptacles, exiting the receptacles with achenes, and flying away with the achenes (see Appendix S2 for a video of interaction; Dryad Digital Repository: 10.5061/dryad.9f5c711). In most cases, wasps flew out of sight immediately, but a few landed on nearby vegetation and dropped the achene to the ground. Another vespoid wasp, *Dolichovespula maculata* (Linnaeus, 1763) was also observed hovering near the openings of spicebush receptacles at the Shasta Lake site, but did not enter the receptacles.

Across all five populations (Table 1), the average orientation of the receptacles was 45° ($SD = 29^\circ$, $n = 484$, Appendix S3, Dryad Digital Repository: 10.5061/dryad.9f5c711).

In the first experiment, in which wasps were allowed to choose between whole achenes and achenes with the marginal organ removed (Table 1, ‘Choice’), between 18 and 101 trials were conducted at each of the eight field sites (Table 1; Fig. 2). At every field site, whole achenes were preferred over achenes with the marginal organ removed ($P < 0.001$; one-sided binomial tests, $H_A = \text{‘greater’}$).

In the second experiment, in which achenes were taken by wasps to the nest (Table 1, ‘Nest’), only a few achenes were recovered from the excavated nests at each site (Sagehen Creek: $n = 5$; Putah Creek: $n = 10$; Kanaka Valley: $n = 1$). In all cases, the provided supply of achenes was located by the wasps within 5 min, and depleted by the time the author returned 5 hr later. In most cases, a wasp took flight with an achene and flew out of sight, suggesting that most wasps were visiting from nests other than the one under direct observation. At all sites, at least one wasp was observed entering the focal nest with an achene, but the nests were not observed continuously throughout the 5 hr time interval, so data were not collected on the fate of each seed taken by a wasp. In all cases, recovered achenes were found at the bottom of the nest (the likely refuse area of the nest

[MacDonald et al. 1975]), and were stripped of their marginal organ (Appendix S4; Dryad Digital Repository: 10.5061/dryad.9f5c711).

In the third experiment, in which wasps were trapped using an extract of the whole achenes, no wasps were trapped in the control traps, while between 11 and 46 wasps were recovered in the traps baited with extract (Butte Creek: 46 wasps; Kaweah River: 21 wasps; Lake Hennessy: 11 wasps; Table 1).

Nutritional analysis revealed that the achene marginal organs from Hooker Creek comprised 21.6% crude protein, 5.9% crude fat, 1.8% water-soluble carbohydrates, 0.5% simple sugars, and 0.3% starch. All of these measurements are on a dry mass basis.

DISCUSSION

Our work provides preliminary evidence that wasps of the genus *Vespula* act as dispersers of *C. occidentalis*. Our survey of wild *C. occidentalis* stands has shown that visitation of *C. occidentalis* receptacles by *Vespula* is a common interaction in the wild (Table 1). Our follow-up choice experiment provided evidence that the marginal organ of the achene is the part of the diaspore attractive to wasps, while nutritional analysis of this organ showed that fat and protein is the likely reward provided by the plant to its dispersers. The fat and protein content of the marginal organ, comparable to that of lean chicken, pork, or beef (Kerth 2013), is consistent with the typical food sources of *Vespula* wasps (Akre et al. 1981), voracious predators and/or scavengers well known for their attraction to high-protein food sources. Compared to other food bodies provided by plants as a reward for insect dispersers (primarily ants), the marginal organ of *C. occidentalis* ranks amongst the highest yet recorded in terms of fat and protein contents (Fischer et al. 2008). Finally, our experiments on volatile extract of the achenes (Table 1), while preliminary, indicate that olfaction plays a critical role in the *Vespula*-*Calycanthus* interaction, likely signaling the presence of the fat and protein reward.

Additional work is clearly needed in order to characterize and test this interaction in more detail, particularly research into other potential dispersers of the achenes (e.g., ants and rodents) and the details of the reward and detection system. Research into the latter subject (Beck et al. 2019) suggests that detection of spicebush achenes by *Vespula* wasps is based on a volatile chemical signature considerably different from the chemical signature recently elucidated in Chinese *Stemona* (Chen et al. 2018), a plant with seeds dispersed by wasps of the Eurasian genus *Vespa*, a close relative of *Vespula*. Interestingly, the complex chemical signature of spicebush achenes included volatiles identified as chemical cues from several other *Vespula* food sources (Beck et al. 2019).

In the course of this research, it was also observed that *V. pensylvanica* visits capsules of pipevine

(*Aristolochia californica* Torr.), a California-endemic relict vine (Stebbins and Major 1965). Observations were made at two of the field sites where pipevine and spicebush co-occur, Cedar Creek (D. Burge 2134, DAV) and Butte Creek (D. Burge 2286, DAV; Table 1). Anecdotal reports of this interaction have appeared on the internet (search of the terms 'pipevine' and 'yellowjacket,' <http://www.google.com>, 21 August 2018), but no peer-reviewed publications appear to have mentioned it. Observations also indicated that wasps visit dehiscing capsules of pipevine and actively remove seeds, taking flight with seeds in the same manner as spicebush. Preliminary observations suggest that the attractive portion of the seed is a strip of soft, spongy tissue filling a longitudinal furrow in the seed (Appendix S5; Dryad Digital Repository: 10.5061/dryad.9f5c711). If *Aristolochia* is included, diaspore dispersal by wasps is now known to occur in five genera, each from a different order (*Aquilaria*, Malvales; *Aristolochia*, Piperales; *Calycanthus*, Laurales; *Stemona*, Pandanales; *Trillium*, Liliales; *Vancouveria*, Ranunculales).

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APPENDIX I

CALYCANTHUS POPULATIONS INCLUDED IN THIS STUDY

For each population, the format is as follows: collector name and number, name of locality, county, GPS coordinates (WGS84 Datum), County, State. Vouchers deposited at the U. C. Davis Center for Plant Diversity (DAV).

Calycanthus occidentalis.—D. Burge 2122, 6 Aug 2016, Butte Creek (GPS: 39.7930, –121.6625), Butte County, CA. D. Burge 2123, 20 Aug 2016, Hooker Creek (GPS: 38.3620, –122.4684), Sonoma County, CA. D. Burge 2125, 25 Aug 2016, Holiday Harbor (GPS: 40.8042, –122.3121), Shasta County, CA. D. Burge 2127, 26 Aug 2016, Shotgun Creek (GPS: 41.0538, –122.3826), Shasta County, CA. D. Burge 2132, 31 Aug 2016, Cedar Creek (GPS: 40.7782, –122.0047), Shasta County, CA. D. Burge 2201, 20 Nov 2016, South Fork Kaweah River (GPS: 36.3961, –118.8810), Tulare County, CA.

APPENDIX II

VESPULA POPULATIONS INCLUDED IN THIS STUDY

For each population (Table 1), the format is as follows: collector name and number, description of locality, county, GPS coordinates, County, State. All voucher specimens deposited at the Bohart Museum of Entomology, U. C. Davis. All collections are workers.

Vespula acadica.—D. Burge 1008, 22 Aug 2016, Sagehen Creek (GPS: 39.434, –120.2588), Nevada County, CA.

Vespula alascensis.—D. Burge 1006, 21 Aug 2016, Hooker Creek (GPS: 38.362, –122.4684), Sonoma County, CA. D. Burge 1013, 27 Aug 2016, Mount Ashland (GPS: 42.0749, –122.7151), Jackson County, OR. D. Burge 1020, 3 Sep 2016, Horse Flat (GPS: 44.7753, –116.5414), Adams County, ID.

Vespula pensylvanica.—D. Burge 1000, 15 Aug 2016, Butte Creek (GPS: 39.793, –121.6625), Butte County, CA. D. Burge 1005, 21 Aug 2016, Hooker Creek (GPS: 38.362, –122.4684), Sonoma County, CA. D. Burge 1007, 22 Aug 2016, Sagehen Creek (GPS: 39.434, –120.2588), Nevada County, CA. D. Burge 1010, 25 Aug 2016, Shasta Lake (GPS: 40.8043, –122.3121), Shasta County, CA. D. Burge 1011, 26 Aug 2016, Shotgun Creek (GPS: 41.0538, –122.3826), Shasta County, CA. D. Burge 1012, 27 Aug 2016, Mount Ashland (GPS: 42.0749, –122.7151), Jackson County, OR. D. Burge 1017, 31 Aug 2016, Cedar Creek (GPS: 40.7783, –122.0048), Shasta County, CA. D. Burge 1019, 3 Sep 2016, Horse Flat (GPS: 44.7753, –116.5414), Adams County, ID. D. Burge 1022, 13 Oct 2016, Putah Creek (GPS: 38.5293, –121.761), Yolo County, CA. D. Burge 1023, 20 Oct 2016, Kanaka Valley (GPS: 38.7534, –121.026), El Dorado County, CA.